Electron microscopic band-interband pattern of polytene chromosomes in *Drosophila nasuta albomicans*

2 Salivary gland chromosome 2L

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Abstract

The band-interband pattern (division 28-52) of salivary gland chromosome 2L in *Drosophila nasuta albomicans* was studied by light (LM) and electron microscopy (EM) using squash preparations and surface-spread polytene (SSP) chromosome preparations, respectively. LM and EM maps were compiled. Based on the digitized EM patterns of five homologous SSP chromosomes a computerized EM chromosome map was plotted. The EM pattern analysis showed a total number of 479 chromosome bands with an almost 83% increase compared with the LM analysis of squash preparations. By extrapolation of the data from 39% of the polytene genome analysed so far in *D. n. albomicans*, a total number of 2,926 chromosome bands was calculated. This is almost the same number of bands as was calculated earlier for *Drosophila hydei* using the same SSP chromosome preparation technique. The data in the literature concerning variations in the number of chromosome bands in different *Drosophila* species, the various chromosome preparation techniques adopted, and the different criteria used for the EM pattern analyses, are discussed.

Introduction

The band-interband pattern of polytene chromosomes has been interpreted by means of the interbands as indicating active or potentially active genes during a specific developmental stage (*cf* Crick, 1971; Speiser, 1974; Zhimulev *et al.*, 1981). Thus, one would expect similar numbers of band-interbands in different *Drosophila* species, especially in strongly related ones owing to their morphological and physiological similarities. However, the total number of chromosome bands found in *D. melanogaster* by EM analyses during the last two decades (*cf* Sorsa, 1988; Sorsa *et al.*, 1984; Saura *et al.*, 1988) indicates almost double the number of bands found by EM analyses in each of the remaining *Drosophila* species.

Therefore, it is still an open question as to whether this difference between *D. melanogaster* and the remaining *Drosophila* species is genetically fixed or mainly due to methodological differences in the various studies. Furthermore, it is not known how much conformity in the number of band-interbands really exists in the remaining *Drosophila* species, even if the methodological differences of chromosome preparation and the different criteria of EM pattern analysis of the various studies could be excluded.

In the present paper, chromosome 2L of *D. n. albomicans* has been analysed on the LM and the EM level, using squash and SSP chromosome preparations, respectively. Photo maps as well as a computerized chromosome map based on the EM analyses of five homologous SSP chromosomes are presented. Together with a recent paper on chromosome 2R in *D. n. albomicans* (Singh and Kalisch, 1991b), we have now analysed about 39% of the total polytene genome. These data together with earlier studies in *D. hydei* (Kalisch *et al.*, 1985, 1986a,b) are the basis of our first attempt to compare the number of band-interbands in different *Drosophila* species by using the same preparation technique and the same criteria of EM pattern analysis.

Materials and methods

The wild type strain *D. n. albomicans* used in the present study was obtained from the National *Drosophila* Species Resource Center, Bowling Green, Ohio, U.S.A. (stock number 15112-1751). Larvae were raised on standard food medium at 25 ± 1°C by feeding additional baker's yeast to obtain an optimized development up to the late third instar.

Techniques of chromosome preparation (squash preparation as well as SSP chromosome preparation) and of microscopic analyses (LM and EM) used in the present work are the same as described in recent papers (Singh and Kalisch, 1991a,b). A computerized EM chromosome map was plotted based on pooled data of chromomeric measurements from micrographs of five homologous SSP chromosomes. The procedure of measurements, digitization, and computerized plotting of the chromosome map are as described in earlier papers (Kalisch *et al.*, 1984, 1986a,b).

Division sectioning and labelling of chromosome 2L is based on the almost homosequential reference map of the subgroup member *D. nasuta nasuta* (Ranganath and Krishnamurthy, 1974). Subdivision sectioning was done according to the needs for plotting a computerized EM chromosome map (Kalisch *et al.*, 1984). Each band in the EM map (Figure 1) is denoted by a dot and the total number of bands counted in each subdivision is given at the right hand margin. A division-wise comparison of the band-interband patterns between the LM analyses of squash preparations and the EM analyses of five homologous SSP chromosomes, including closely juxtaposed bands of similar thickness and type (doublets and triplets; with interbands not longer than 0.094 μm; Kalisch *et al.*, 1986b) are shown in Table 1.

Results

Chromosome 2L is the second smallest polytene complement (next to the microchromosome or 4th chromosome) in *D. n. albomicans*. It represents about 17% of the total length in salivary gland chromosomes. The two prominent band groups of subdivision 28B at the tip of the chromosome can be used as landmarks. At the proximal end, the characteristic cytology of division

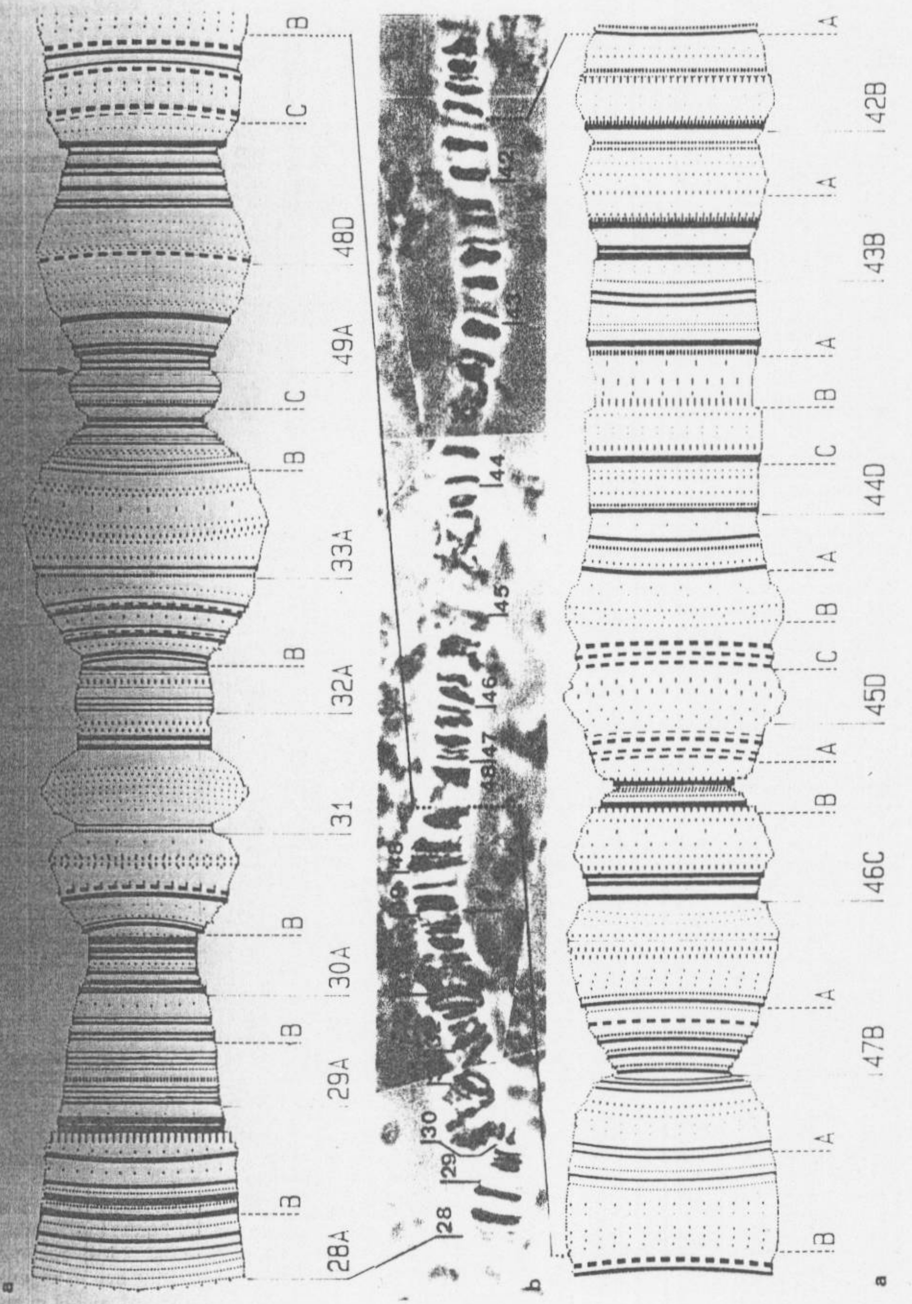

Figure 2 See next page.

Table 1 Band-interband pattern of salivary gland chromosome 2L in *Drosophila nasuta albomicans*: division 28-52

Divisions and sub-divisions	LM map t	EM chromosome map: D	S	t	%
28 A	4	–	7	7	
B	4	1T + 1	4	9	
Totals	8	1T + 1	11	16	100.0
29 A	4	1	8	10	
B	3	2	1	5	
Totals	7	3	9	15	114.3
A	6	1T + 1	3	8	
B	4	2	3	7	
Totals	10	1T + 3	6	15	50.0
31	5	1	5	7	
Total	5	1	5	7	40.0
32 A	3	2	3	7	
B	4	3	3	9	
Totals	7	5	6	16	128.6
33 A	4	2	4	8	
B	5	1T + 1	4	9	
C	4	1	3	5	
Totals	13	1T + 4	11	22	69.2
34 A	3	1	5	7	
B	4	1T + 1	4	9	
Totals	7	1T + 2	9	16	128.6
35 A	3	1	4	6	
B	6	2	8	12	
Totals	9	3	12	18	100.0
36 A	3	1T + –	3	6	
B	4	1T + 1	3	8	
Totals	7	2T + 1	6	14	100.0
A	5	1	7	9	
B	4	1	7	9	
Totals	9	2	14	18	100.0
38 A	4	1T + 1	5	10	
B	5	2	6	10	
C	4	1	4	6	
D	4	1	5	7	
Totals	17	1T + 5	20	33	94.1
39 A	5	1	6	8	
B	4	1	5	7	
C	7	2	8	12	
Totals	16	4	19	27	68.8
40 A	4	2	4	8	
B	4	1	6	8	
Totals	8	3	10	16	100.0

Table 1 (Continued)

Divisions and sub-divisions	LM map	EM chromosome map:			
	t	D	S	t	%
41 A	5	1T + –	5	8	
B	5	1	5	7	
Totals	10	1T + 1	10	15	50.0
42 A	6	2	5	9	
B	5	2	4	8	
Totals	11	4	9	17	54.5
43 A	5	1	5	7	
B	5	1	5	7	
Totals	10	2	10	14	40.0
44 A	5	1	5	7	
B	3	–	3	3	
C	3	–	3	3	
D	1	1	2	4	
Totals	12	2	13	17	41.7
45 A	4	–	5	5	
B	2	–	5	5	
C	2	–	3	3	
D	2	–	4	4	
Totals	10	–	17	17	70.0
46 A	3	1	3	5	
B	4	2	–	4	
C	4	1T + 1	2	7	
Totals	11	1T + 4	5	16	45.5
47 A	5	1	7	9	
B	4	3	2	8	
Totals	9	4	9	17	88.9
48 A	3	1T + –	4	7	
B	4	–	8	8	
C	3	1	4	6	
D	5	4	4	12	
Totals	15	1T + 5	20	33	120.0
49 A	6	2	8	12	
B	3	1	5	7	
C	3	1	5	7	
Totals	12	4	18	26	116.7
50 A	4	1T + –	5	8	
B	4	1	6	8	
C	5	1	5	7	
D	3	1	3	5	
Totals	16	1T + 3	19	28	75.0

Table 1 (Continued)

Divisions and sub-divisions	LM map	EM chromosome map:			
	t	D	S	t	%
51 A	2	1	1	3	
B	3	2	2	6	
C	4	1	5	7	
D	2	—	4	4	
Totals	11	4	12	20	81.8
52 A	3	1T + 1	3	8	
B	2	—	4	4	
C	3	—	6	6	
D	3	2	4	8	
Totals	11	1T + 3	17	26	136.4
Totals 28–52	261	12T + 73	297	479	83.5

Comparison between the LM analysis of the original chromosome squash preparation in Figure 1 and the data of the EM chromosome map depicted in Figure 2, which are based on the EM analyses of five homologous SSP chromosomes. T, number of triplet bands; D, number of doublets; S, number of single bands; t, total number of bands; and %, percentage increase of additional bands in the EM chromosome map.

2 (flanked by the prominent band groups of subdivision 52A and 52D) favours identification. These landmarks appear with a comparable cytological prominence in both squash as well as SSP chromosome preparations (Figures 1a and 1b).

Division 31 appears folded in SSP chromosomes, probably due to ectopic pairing affinities among different polytene structures within the division (Figure 1a). This landmark is hardly visible in squash preparations. Even in SSP chromosomes, its appearance varies in the homologous chromosomes of the same salivary gland preparation. This cytological peculiarity is somewhat similar to a loop formation of subdivision 53 in 2R, which is based on strong ectopic pairing between different chromosome sections. However, the loop in subdivision 53 is permanently found in squash preparations and in almost all SSP chromosomes (Singh and Kalisch, 1991b,c).

Chromosome 2L of *D. n. albomicans* possesses a homosequential band-interband pattern similar to that of *D. n. nasuta*, except for a paracentric inversion of region 34A-49A. The break-points of the inversion (marked by arrows in the LM and the EM map of Figures 1 and 2) were confirmed by analysing the heterozygous loop formed in *D. n. albomicans* x *D. n. nasuta* F1-hybrids. Based on the assumption that *D. n. nasuta* is older in evolutionary phylogeny than *D. n. albomicans* (Hägele and Ranganath, 1982; Ranganath and Hägele, 1982), we considered the band-interband pattern of *D. n. nasuta* as a reference for mapping purposes. Thus, the inverted chromosome segment in *D. nasuta albomicans* was labelled, following the pattern labelling in the *D. n. nasuta* LM map (Ranganath and Krishnamurthy, 1974).

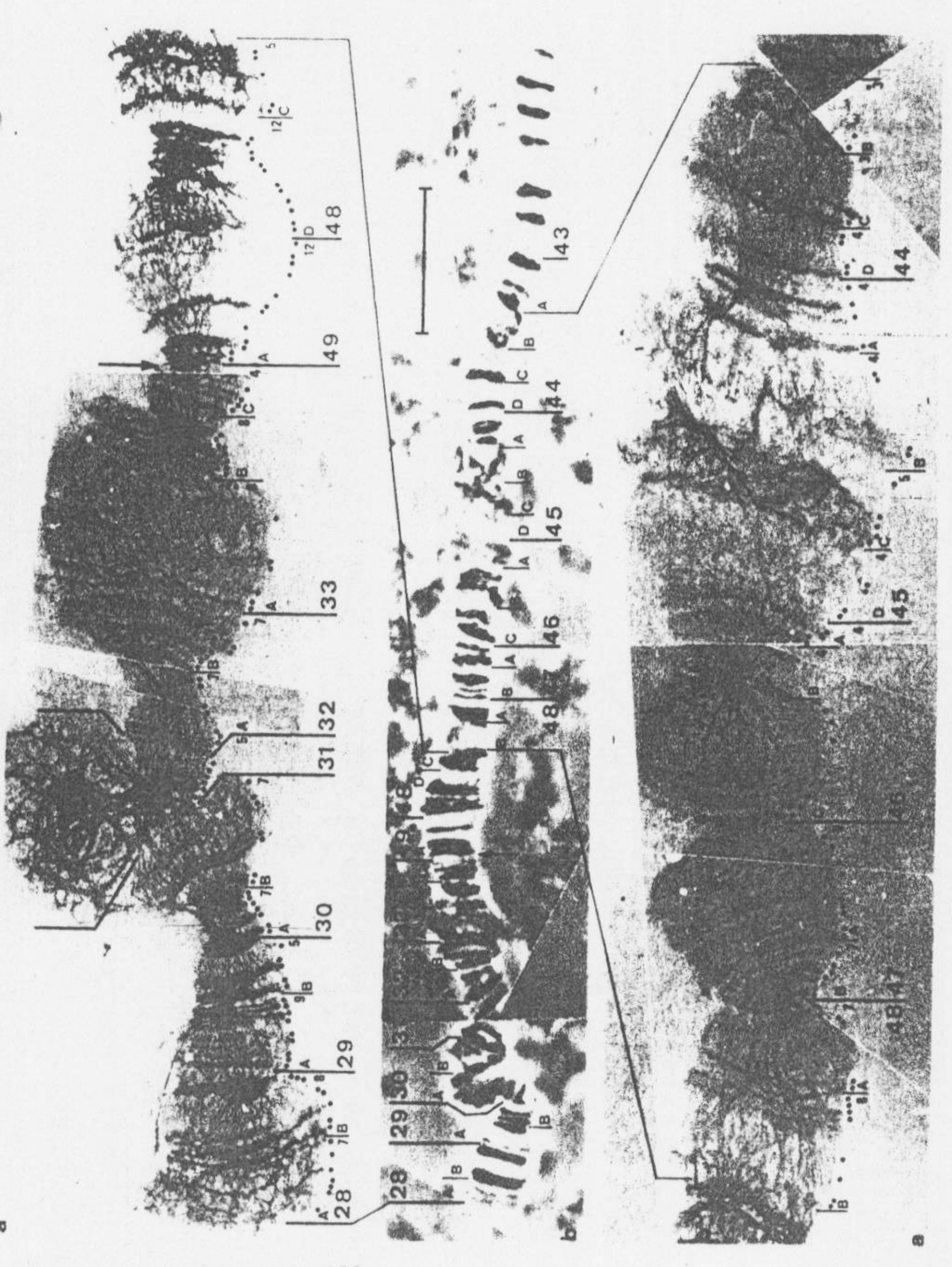

Figure 1 See legend on page 102.

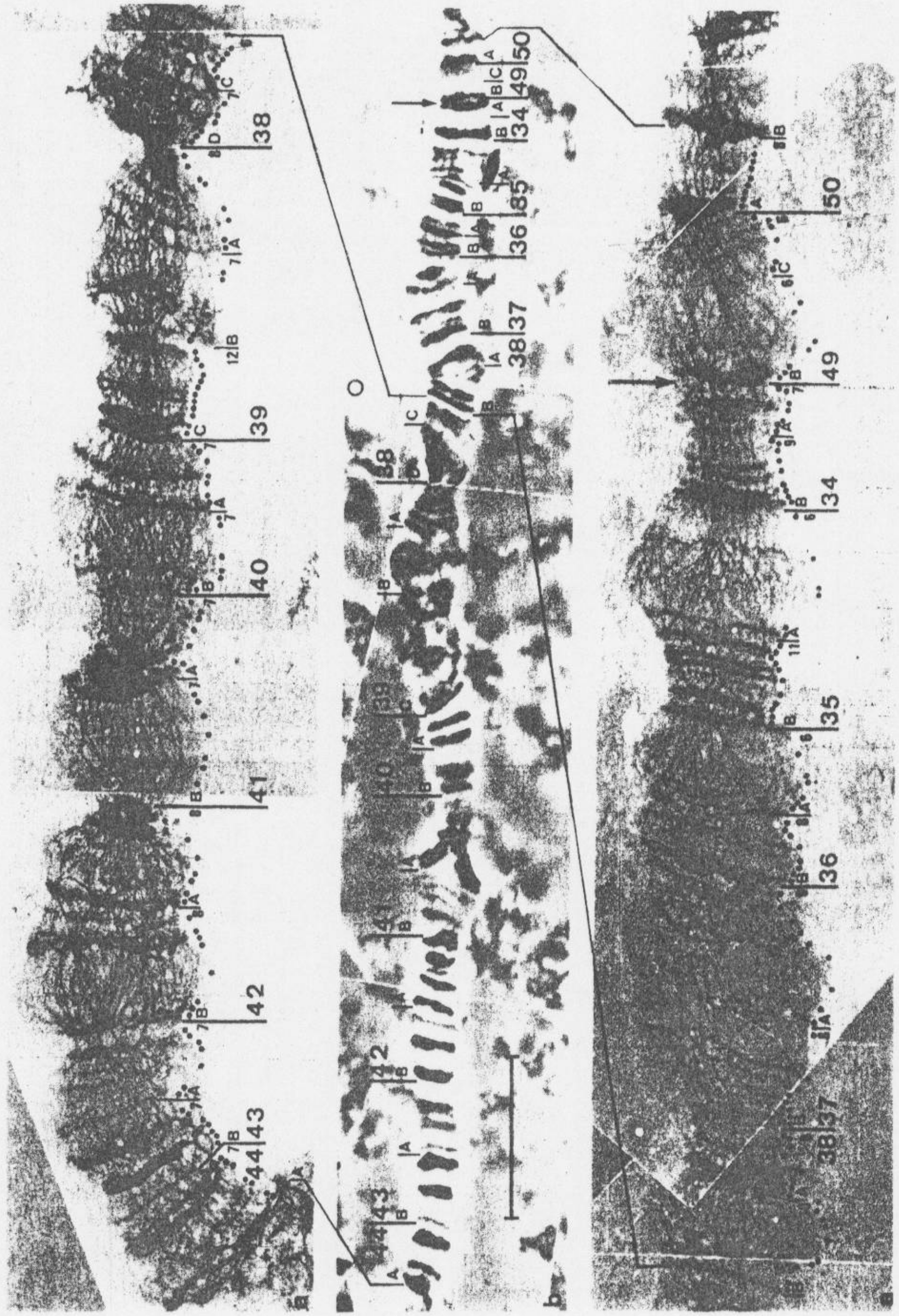

Figure 1 Continued

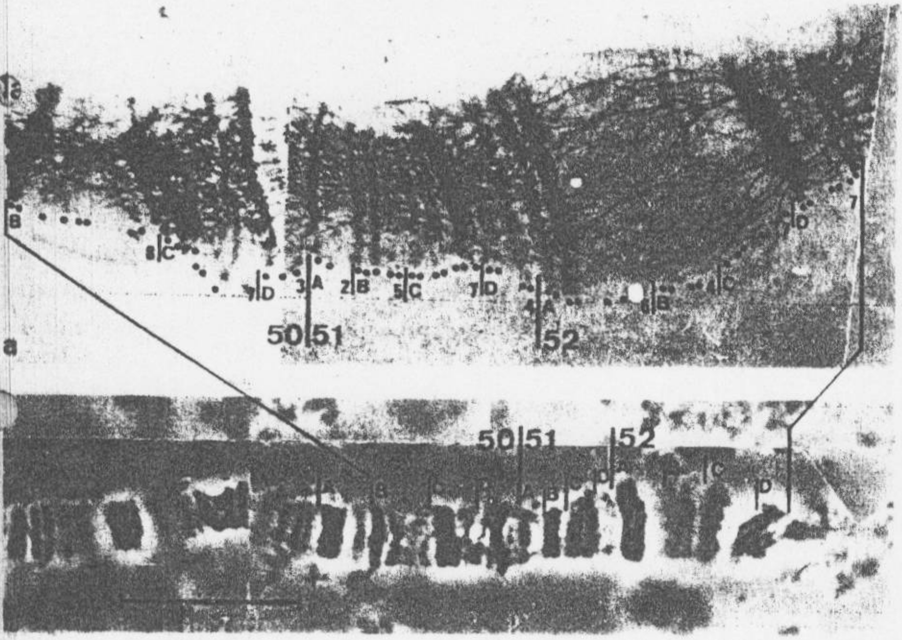

Figure 1 EM (a) and LM (b) maps of chromosome 2L (division 28-52) in *Drosophila nasuta albomicans* arranged in three consecutive plates (28A-44B; 44A-50A: 50B-52D). All micrographs used in the EM map come from a single SSP chromosome. In the 44A-50A plate of the LM map, the inverted subdivisions 38A-34A together with subdivisions 49A-50A are replaced by a micrograph from another squash preparation. Division sectioning is based on the LM reference map of *D. n. nasuta* (Ranganath and Krishnamurthy, 1974). Arrows indicate the break points (49A-34A) of the inverted chromosome region. Each dot in the EM map labels one chromosome band identified in the original EM micrographs. The total number of bands in the EM map are given at the right border in each subdivision. The bar scales equal 10 μm in the LM map and in the surface-spread chromosomes of the EM map.

Wilson *et al.* (1969) suggested that members of the *D. nasuta* subgroup differ from each other by fixed paracentric inversions. The inversion referred to as 2LA in their LM study seems to correspond to the inversion observed in the present study. Comparison of our LM map with that of Lin *et al.* (1974) also indicates a similar band-interband pattern in chromosome 2L.

The average length and width of chromosome 2L in squash preparations are about 135 μm and 3.5 μm, respectively. In SSP chromosome preparations with a comparable degree of polyteny, the average data are 375 μm in length and 11.4 μm in width, indicating an almost three-fold longitudinal (x2.8) and lateral (x3.3) degree of spreading.

Number of chromosome bands

The total number of bands is not mentioned anywhere in previous LM studies dealing with the band-interband pattern of polytene chromosomes and in the photo maps of *D. n. nasuta* as well as *D. n. albomicans* (Wilson *et al.*, 1969; Sajjan and Krishnamurthy, 1973; Lin *et al.*, 1974; Ranganath and Krishnamurthy, 1974; Roy and Lakhotia, 1981). Its assessment from published micrographs is difficult, because the analysis of an individual published micrograph generally reveals fewer bands compared with those seen in a detailed microscopic analysis of the original chromosome preparation.

In the present study, 261 chromosome bands in the LM analysis of squash preparations (Figure 1b, and Table 1) were counted. The EM map of chromosome 2L in Figure 1a revealed a total of 454 bands. An additional 25 bands were found by EM analyses of another four homologous SSP chromosomes. These 479 bands (83% increase compared with the LM analysis) are included in Table 1 and in the computerised EM chromosome map (Figure 2a).

LM and EM maps

Chromosome 2L of *D. n. albomicans* was divided into 25 divisions (28-52) according to the *D. n. nasuta* reference map (Ranganath and Krishnamurthy, 1974). Each division was further divided into four subdivisions, depending on its size, the number of polytene structures in it, and the distribution of prominent bands. Division 31 in SSP chromosomes was not divided into subdivisions because of its cytological peculiarity.

LM and EM maps of chromosome 2L in Figure 1 are compiled in three consecutive plates (28A-44B; 44A-50A; 50A-52D). All micrographs used for the EM map come from a single SSP chromosome. In the LM map, the inverted subdivisions 38A-34A together with subdivisions 49A-50A (in plate 44A-50A) are replaced by a micrograph from another squash preparation. The chromosomes in the LM and the EM map represent comparable degrees of polyteny. Both maps are presented at the same magnification. The higher resolution and larger size of the polytene structures in the EM map are the result of electron microscopy and the SSP chromosome preparation technique, respectively (Kalisch and Whitmore, 1986).

Computerized EM chromosome map

Band and interband lengths recorded from the micrographs of five homologous SSP chromosomes were digitized according to a method described previously (Kalisch *et al.*, 1984; Singh and Kalisch, 1991b). The average values were plotted as an EM chromosome map (Figure 2a) which contained 479 bands. The different morphology and chromatin content of the bands were depicted by using dotted and continuous lines of different thickness as well as combinations of both for exceptional morphology (*e.g.* chromosome band 30B6, 42B8, 43B3, and 52D1 in Figure 2a). The LM micrographs were arranged and mapped along with the computerized chromosome map for a direct

Discussion

Comparison between our LM and EM assessments of the chromosome bands shows an increase of about 83% in the EM study (Table 1). A comparable increase (about 98%) was found in a recent study of chromosome 2R in the same species (Singh and Kalisch, 1991b). Generally, the increase of chromosome bands in a revised map is based on the quality of the orginal LM map used. LM reference maps revised several times by different authors naturally include more details than maps which were originally established for only taxonomic purposes (Martin, 1974). A 40% increase was found in the EM analyses of SSP chromosomes in *D. hydei* (Kalisch *et al.*, 1985, 1986a,b) as well as in *D. subobscura* (Kalisch and Böhm, 1985), whereas a 220% increase was evident in *Chironomus tepperi* (Kalisch and Whitmore, 1983).

Including the 662 bands of chromosome 2R from the earlier study in *Drosophila nasuta albomicans* mentioned above (Singh and Kalisch, 1991b), the two chromosome complements (2L and 2R), which represent about 39% of the euchromatic length of polytene chromosomes, possess 1,141 bands. By extrapolation, the total number in *D. n. albomicans* would be about 2,926 chromosome bands, if the same technique of pattern analysis were used for the remaining chromosomes.

The calculation of total band-interband numbers by extrapolation may be connected with a certain percentage error (Kalisch and Whitmore, 1983). However, our analyses in *D. hydei* indicated that extrapolation of the EM data of SSP chromosomes from about 40% of the genome produced reasonable results as we found in a comparison between our extrapolated data and the EM data of a photo map for the whole polytene. In both analyses, there was a total number of about 2,900 to 3,000 band-interbands for *D. hydei* (Kalisch *et al.*, 1985, 1986a,b).

Similarity in the band-interband numbers in both species analysed by the same technique may be considered as in accordance with the assumption that the interbands represent constantly active or potentially active genes whose products are required mainly for the general physiological maintenance of the individual (housekeeping genes). Therefore, in species possessing similar levels of morphological and physiological complexities, one would not expect a substantial difference in the total number of chromomeres if the general architecture of the band-interband pattern is the same.

These assessments hardly fit with the total number of about 5,000 chromosome band-interbands, counted in the entire polytene genome of *Drosophila melanogaster* by LM as well as EM analyses (*cf* Lefevre, 1976; Saura *et al.*, 1988; Sorsa, 1988; Sorsa *et al.*, 1984). Even if the number of band-interbands is basically the same in different *Drosophilia* species, one would expect some variations owing to the unequal mean DNA content per chromomere (Kiknadze *et al.*, 1976), differential (developmental, tissue specific, and other) gene activities (Zhimulev *et al.*, 1981), and different levels of polyteny (Singh, 1991). However, a difference of about 2,000 bands between *D. melanogaster* and other *Drosophila* species is substantial and can hardly be counter-argued by the reasons previously stated.

The number of chromosome preparations analysed in *D. melanogaster* was very large in comparison with that analysed in any other species. Our analyses of SSP chromosomes in a species were usually based on five chromosome preparations, whereas Sorsa's (1988) analyses were based on fifty or more chromosome preparations. This naturally leads to more chromosome bands by observation of a larger number of somewhat different developmental band-interband patterns (during the third larval instar) and to a more authentic observation of rarely found polytene structures.

Different interpretations of the very faint, tight, and rarely found polytene structures seem to be another important reason for the varying numbers of chromosome bands. If one compares the faint polytene structures interpreted as chromosome bands in previous work (*op. cit.*) and in our group, we have obviously counted fewer polytene structures as chromosome bands in our studies. Indeed, our interpretation is based on the assumption that in SSP chromosomes a band represents itself by a polytene structure in all or at least in most of the homologous chromatid sections, whereas a polytene structure only represented by a few of the homologous chromatid sections is to be seen as an occasional configuration. These different kinds of interpretation are based on the two types of chromosome preparation techniques used. In the thin sectioning preparation, only a part of the chromatids were seen, whereas all the chromatids of a polytene chromosome were found in every SSP chromosome preparation. On the other hand, both preparation techniques revealed comparable resolutions in pattern analysis (Bröer, 1986).

The band-interband pattern analysis of *D. melanogaster* by the Sorsa (*op. cit.*) group is so far the most detailed pattern analysis in existence. However, it cannot be used for a comparison with those of other *Drosophila* species. The analyses of *D. n. albomicans* and *D. hydei*, using the same preparation technique and the same criteria of pattern analysis, indicate a lower number of chromosome bands, but they are better suited for a species-specific comparison. This does not exclude, on the other hand, that a comparable number of bands will be found even in *D. nasuta albomicans* and *D. hydei*, if a comparable number of preparations and the same criteria of pattern analysis were used. Therefore, it is suggested that there is no genetically fixed difference in about 2,000 bands between *Drosophila melanogaster* and *D. n. albomicans* and *D. hydei*.

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